AATD 2023 MEETING

Molecular Therapies for Liver Disease in Alpha-1 Antitrypsin Deficiency

Abstract Book

Pozzuoli (NA), Italy
September 7th - 8th, 2023
Telethon Institute of Genetics and Medicine (TIGEM)

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Alpha1-antitrypsin deficiency: a forgotten liver disease

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Alpha1-antitrypsin deficiency (AATD) arises due to mutations in alpha1-antitrypsin (AAT) gene that interfere with production and/or secretion of this protein produced primarily in hepatocytes. The most common variants are Pi*Z and Pi*S and the homozygous occurrence of the former (Pi*ZZ genotype) is the predominant cause of severe AATD. Pi*ZZ subjects typically accumulate AAT in hepatocytes and may present with an cholestatic disorder in early childhood or develop significant liver fibrosis in later adulthood. While Pi*ZZ harbor ~20x increased risk of liver cirrhosis, individuals with heterozygous Pi*Z mutation (Pi*MZ genotype) possess only display only twice increased predisposition and exhibit advanced liver fibrosis primarily in presence of additional risk factors. Recent studies defined the histologic presentation of AATD-related liver disease and uncovered several non-invasive tools that can be used for patient stratification. The increasing knowledge about the disease phenotype together with its well-defined pathomechanism as monogenic, proteotoxic disorder led to development of multiple drug candidates that either decrease AAT production or promote AAT secretion from the liver. Among the former, the small interfering RNA (siRNA) fazirsiran demonstrated promising results as well as a reassuring safety profile in two phase 2 clinical trials, while the siRNA belcesiran is being currently tested. In addition to that, secretion promoting candidates have been/are currently tested in several phase 2 clinical trials, but a conclusive evidence of their efficacy is currently missing. Several other genetic approaches using gene/RNA editing are in late pre-clinical or early clinical studies and make AATD a coveted target of modern pharma. While the future looks bright, several challenges remain, such a better understanding of the natural disease course, a discovery and validation of disease biomarkers as well as agreement on endpoints needed for approval of current drug candidates.

Increased liver fibrosis and serum Z polymer levels are associated with increased risk of severe liver disease outcomes in a prospective cohort of adults with alpha-1-antitrypsin deficiency

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Background: Outcomes of adults with ZZ alpha-1-antitrypsin deficiency (AATD) liver disease is variable and unpredictable. There is a lack of prospective data, including on the utility of liver biopsy. Hypothesis: Prospective clinical and biopsy data will identify factors associated with severe liver disease outcomes in AATD.
Objective: Use data from a prospective, multi-center adult cohort of AATD ZZ subjects with protocol enrollment liver biopsies to identify the prognostic value of clinical markers and biopsy for the development of severe liver disease outcomes.
Methods: Homozygous ZZ AATD adults enrolled prospectively at 3 US sites with standardized clinical evaluations, followed annually with standardized data collection and outcomes recorded. Liver biopsy obtained at enrollment, unless previous biopsy confirmed cirrhosis (grouped as Ishak >4). Fibrosis scored using Ishak score (stage 0, stage 6). Minimal fibrosis defined as Ishak 0-1, increased fibrosis as Ishak > 2. Severe liver disease outcomes defined as death related to liver disease, liver transplantation or listing.
Results: 96 subjects enrolled; 72% minimal fibrosis and 28% increased fibrosis. 62% had normal FEV1 (>80% predicted), with 38% on AAT protein replacement. 7 severe liver disease related outcomes were reported in median 3.8 years of follow up; 3 liver disease related deaths, 3 liver transplants and 1 on transplant waiting list. Increased fibrosis on enrollment biopsy was significantly associated with these events. (100%, vs 22.5% p< 0.001). AST < 39U/L at enrollment correlated to absence of events (Œ∫= 0.48; p= 0.0047). Baseline fibrosis assessment scores FIB 4 and APRI were higher in those with severe events (FIB 4 7.79 ±5.35 vs 1.47 ±0.80; p= 0.0205; APRI 2.3±1.83 vs 0.3±1.9; p= 0.0286). Negative predictive value of normal FIB4(<1.3) and APRI (<0.5) in predicting severe liver disease outcome was 100%. Higher serum Z protein polymer levels at enrollment were associated with future severe liver outcomes (19.6 ± 9.1µg/ml vs 11± 6.3 µg/ml; p=0.0038). ALT, GGT, elastography scores, FEV1, smoking and alcohol consumption patterns were not associated with adverse liver outcomes.
Conclusion: Increased fibrosis on liver biopsy in patients with AATD is associated with increased severe liver disease outcomes. Non-invasive tests (FIB 4, APRI) and higher Z polymer levels are associated with severe events. We suggest close follow up of ZZ patients with indications of liver disease.

Alpha-1 antitrypsin deficiency associated liver disease – Natural history data gaps and a proposal to overcome them

Ed G. Marins

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Alpha-1 anti-trypsin deficiency (AATD) is found in diverse populations and may be one of the most common single-locus genetic diseases in the world. It is estimated that 1 in 2000-5000 newborns in Europe and North America have the Pi*ZZ genotype1. A 2012 analysis by De Serres et al. in a total population of 5,264,150,044 across 97 countries found that 190,574,275 (1:27.6) were carriers of at least one of the S or Z SERPINA1 alleles associated with AATD and 5,468,848 (1:963) were carriers of two alleles (ZZ, SZ, or SS), of whom 181,894 (0.1%) were Pi*ZZ, 1,269,054 (0.7%) were Pi*SZ and 4,017,900 (2.1%) were Pi*SS2. A total of 42,564,136 (22.3%) individuals were Pi*MZ while the remaining 142,541,291 (74.8%) were Pi*MS2.

Despite the relatively high number of affected individuals in the context of a rare disease and the many initiatives to better understand the natural history of the AATD associated liver disease such as the EASL/ERN cohort, the EARCO international AATD registry and the Swedish birth cohort among others, many data gaps in the understanding of the disease trajectory remain. With this challenge in mind, a strategy to address some of these data gaps is proposed and intends to create a consortium between registries, academia and industry in order to standardize data collection among participants and provide a larger cohort of AATD patients through data pooling. This will enable further characterization of the natural history of the liver and lung diseases associated with AATD and their interplay, as well as the collection of patient-reported outcomes and health-care utilization costs that are currently missing in this population.

Characteristics of Patients with Alpha-1 Antitrypsin Deficiency Highlighting Liver Symptomology Among the United States Cohort in the Alpha-1 Foundation Research Registry

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Introduction: Alpha-1 antitrypsin deficiency (AATD) is a rare inherited condition that leads to increased risks of developing lung and liver disease, however the burden of liver disease in a general cohort of mixed genotype AATD patients needs further exploration. Since 2019, the Alpha-1 Foundation (A1F) has collected ongoing patient reported and clinical data, including lung and liver disease symptomology, that will allow for the longitudinal study of AATD.
Methods: A HIPAA compliant online platform, Research Electronic Data Capture (REDCap), was utilized to collect over 1,300 discrete data points per patient between June 2019 to June 2023. Key leaders in the Alpha-1 community provided input in developing questionnaires. Data collected include demographics, clinical history, and quality of life questions. Recruitment was facilitated through communications from A1F and AlphaNet at virtual and in-person events, and from the historical contact registry at Medical University of South Carolina.
Results: The Alpha-1 Research Registry has enrolled 3,076 participants, from all 50 states. Participants identify largely as female (67%), white (97.5%), and with a mean age of 54.2 +/- 15.3 years (range <1-90 years). Deficient genotypes account for more than 50% (n=2341) including 40% PiZZ, 11% PiSZ, and 3% other rare deficient alleles. AATD testing was reported (n=2264) because of lung symptoms (44%), family history (32%), liver symptoms (10%), incidental findings on direct-to-consumer testing (7%) and other reasons (7%). Among those who reported AATD testing due to liver symptoms, we found 43% to be PiZZ, 19% PiMZ and 15% PiSZ. Thirty seven percent (n=2204) reported to be liver affected, of which fatty liver disease was reported in 24%. For Registry participants less than 18 years (n=41), 46% reported a history of childhood jaundice, 61% reported abnormal liver function testing and 10% diagnosed with cholestasis.
Conclusion: This successful comprehensive research platform housed within the A1F patient advocacy organization exhibits a range of genotypes with various clinical presentations. This highlights the need to further characterize liver disease in AATD patients with a robust Natural History Study. Such a study would provide useful information in the design of therapeutic clinical trials. Furthermore, the Alpha-1 Research Registry is presently utilized to identify and invite suitable participants for clinical trials and research studies. Currently, eligible Registry participants can participate in a NIH- and A1F-sponsored Longitudinal Biomarker Consortium, an initiative to determine if biomarkers can predict disease prognosis and outcome.

Alpha-1-Antitrypsin Deficiency: Lessons Learned from the Childhood Liver Disease Research Network (ChiLDReN)

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Alpha-1-Antitrypsin Deficiency (A1ATD) is a frequently undiagnosed genetic disorder with the potential for significant liver-related morbidity and mortality. ChiLDReN, a multi-center NIH-funded prospective study, aimed to elucidate the complexities of A1ATD-associated liver disease in individuals aged birth- 25 years. Data was obtained from LOGIC (A longitudinal study of genetic causes of intrahepatic cholestasis-Clinical Trials identifier NCT00571272). LOGIC is still enrolling but this abstract describes 2 prior analyses of specific patient cohorts.

The study period spanned November 2008-October 2012, enrolling 269 subjects, 208 exhibited native livers and 61 had undergone liver transplants. This baseline analysis of the A1ATD liver disease cohort unveiled a high prevalence of portal hypertension (PHT). Notably, 29% of those with native livers had evidence of PHT. Interestingly, no significant age-related differences were observed between subjects with mild or severe disease. Jaundice, a common clinical feature, was more prevalent in the severe disease group (76%) than in the mild group (57%), marking a significant distinction. Liver function tests highlighted notable differences in bilirubin, ALT, AST, INR, and GGTP levels between subjects with and without PHT.

Despite identifying PHT as a common manifestation, diagnosing it based on chemistries proved challenging due to overlapping results between groups. However, the study underlines the significance of PHT identification, as it can influence patient management and outcomes. The ChiLDReN study underscores the impact of A1ATD on young patients with native livers, emphasizing the necessity for early detection and management.

A related longitudinal analysis explored neonatal cholestasis as a predictor of future PHT. This evaluation included 350 participants (60% male) aged 0-25 years with a native liver. Of these, 278 entered the cohort without PHT, 18 developed PHT during follow-up, and 30 underwent liver transplantation. While neonatal cholestasis was not a strong predictor of subsequent severe outcomes, its association with PHT was noteworthy. The study's longitudinal approach highlighted the slow progression to liver transplantation and rarely death.

In conclusion, the ChiLDReN study provided invaluable insights into A1ATD-associated liver disease in childhood. The findings shed light on the prevalence and significance of PHT, underscored the challenges in diagnosing PHT based solely on chemistries, and emphasized the importance of early detection and management. Furthermore, the study questioned the role of neonatal cholestasis as a definitive predictor of severe disease, highlighting the ongoing risk of complications and progression to severe liver disease throughout childhood..

Prediction of liver-related endpoints by non-invasive fibrosis tests in a longitudinal study of adults with severe alpha-1 antitrypsin deficiency (Pi*ZZ genotype)

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Aims and objectives: Homozygous Pi*Z mutation (Pi*ZZ genotype) confers a strong predisposition to lung and liver disease. While phase 2 clinical trials suggested that treatment with small interfering RNA may improve Pi*ZZ-associated liver phenotype, very little is known about the natural disease course as well as factors predicting the development of liver-related endpoints. To change that, we evaluated risk factors and the predictive utility of non-invasive tests in the multi-center European Pi*ZZ liver cohort.
Methods: 546 Pi*ZZ subjects without concomitant liver diseases or pathological alcohol consumption received a baseline clinical, laboratory, and elastographic assessment. 491 of them had a detailed follow-up interview at least 10 months after their baseline examination.
Results: During a median follow-up of 3.7 years, 31 Pi*ZZ individuals deceased. The main causes of fatality were lung and liver disease, accounting for 11 and 12 deaths, respectively. 11/7 individuals received a lung/liver transplant. 26 Pi*ZZ subjects who developed a hepatic endpoint (liver transplant/death, or decompensated cirrhosis) presented with significantly higher BMI (28 vs. 24 kg/m2, p=2.7x10-4), liver stiffness measurement (21 vs. 5 kPa, p=2.5x10-15), AST-to-platelet ratio index (APRI, 1.0 vs. 0.3 units, p=2.3x10-13), fibrosis-4 index (3.7 vs. 1.3, p=6.5x10-12), and liver enzymes in their baseline examination. Cox regression analysis revealed LSM ≥9.4 kPa (aHR 69.3, 95% CI 16.2-296.7, p=1.1x10-8) and APRI ≥0.6 units (aHR 46.1, 95% CI 13.5-157.5, p=1.0x10-9) as strong predictors of liver-related endpoints. Notably, 366 individuals with LSM<7.1 kPa at baseline did not develop any hepatic endpoint during their follow-up.
Conclusions: LSM and APRI accurately stratify Pi*ZZ individuals according to their risk of liver-related events. Thus, these non-invasive tests may allow risk stratification in clinical practice as well as selection for clinical trials..

RNA base editing for the treatment of Alpha-1 antitrypsin deficiency

Paloma H. Giangrande, Monian P, Shivalila C, Lu G, Bowman K, Bylsma M, Byrne M, Cannon M, Desai J, Faraone A, Favaloro F, Ghosh A, Godfrey J, Hernandez N, Iwamoto N, Kawamoto T, Kumarasamy J, Kandasamy P, Lamattina A, Lemaitre M, Lindsey A, Liu F, Looby R, Luu K, Metterville J, Paik I-H, Pan Q, Pu T, Purcell-Estabrook E, Rheinhardt J, Shimizu M, Singh K, Standley A, Thomas C, Tripathi S, Yang H, Yordanoff R, Yin Y, Yu H, Narayanan P, Vargeese C

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A point mutation in the SERPINA1 gene (G-to-A mutation, PiZ allele) is the most common cause for Alpha-1 antitrypsin deficiency (AATD). The resulting E342K amino acid change leads to misfolding and aggregation of mutant Z-AAT protein in hepatocytes and a decrease in functional wild-type AAT (M-AAT) in circulation. Thus, this single mutation leads to both toxic gain-of-function and loss-of-function phenotypes, leading to progressive liver injury, lung injury, or both and culminates in end-stage liver and pulmonary disease. Current standard of care, which addresses only the lung manifestation, aims to restore serum M-AAT levels to an anticipated therapeutic threshold (11 µM) by weekly intravenous protein augmentation therapy with plasma-purified AAT.
While there are multiple approaches in development to treat AATD, they either address only a subset of patient needs (e.g., silencing approaches to reduce liver aggregates) or do not restore M-AAT (e.g., small-molecule approaches). We aim to decrease Z-AAT and restore M-AAT protein by correcting the PiZ mRNA with AIMers,îÄchemically modified oligonucleotides that direct A-to-I base editing in RNA. Our AIMers incorporate phosphoryl-guanidine (PN) chemistry on a stereopure backbone and recruit endogenous adenosine deaminase acting on RNA (ADAR) enzymes. This approach is expected to preserve physiological regulation of M-AAT while protecting the lungs and decreasing Z-AAT protein aggregation in liver.
To facilitate delivery to hepatocytes, we conjugated AIMers to N-Acetylgalactosamine (GalNAc). In the NSG-PiZ mouse model that expresses the human PiZ allele, GalNAc-AIMers direct significant PiZ editing in hepatocytes and durably increase serum AAT protein levels. With loading dose followed by bi-weekly dosing, serum AAT levels are maintained at concentrations >11 µM and reach a peak concentration of ~20-30 µM. Secreted AAT protein has the expected wild-type amino acid sequence and inhibits neutrophil elastase. Over time, treated mice show a decrease in liver inflammation and hepatocyte turnover, and reduction in the size of PAS-D positive globules. Lastly, we show that GalNAc-AIMers support dose-dependent PiZ mRNA base editing in vitro in primary and iPSC-derived human hepatocytes deficient for M-AAT (MZ heterozygous and ZZ homozygous lines). These findings highlight the potential of GalNAc-AIMers as a therapeutic approach to address both liver and lung manifestations of AATD.

RNA Editing for Alpha-1 Antitrypsin Deficiency

Leah Liu, Boulay D, Brown C, Butler D, De Silva D, Gottschalk S, Flum J, Hu S, Jenness D, Levandowski W, Maciejewski M, Pink M, Pires E, Popovici-Muller M, Putta M, Saha A, Shadid M, Su K, Ulkoski D, Wantz A, and Erion D.

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RNA editing is a natural physiological process that occurs in cells where a specific single base edit is mediated by an enzyme called Adenosine Deaminase Acting on RNA ('ADAR'). Korro Bio's RNA editing approach involves co-opting this endogenous editing system via a proprietary engineered oligonucleotide to introduce precise edits to RNA. ADARs bind double-stranded RNA structures and convert a single base of Adenosine ('A') on RNA, into an Inosine ('I') that is commonly translated as a Guanosine ('G'), using an enzymatic process. An RNA editing approach for Alpha-1 Antitrypsin Deficiency patients with the Z allele can address the E342K (G>A) mutation responsible for both lung and liver-associated disease. Korro Bio has achieved dose dependent RNA editing with novel oligonucleotides in patient-derived human liver-like cells and M/Z primary hepatocytes. In addition, Korro Bio's Oligo A has demonstrated 63% editing in the PiZ mouse model which expresses the human Z allele. In sub-chronic studies, Oligo A increased circulating WT-A1AT and reduced the amount of Z-A1AT in the liver. Translation to non-human primates (NHP) was demonstrated using a specifically designed oligo for the endogenous coding region of the cyno SERPINA1, introducing a novel mutation which can be detected in circulation. In NHP, editing of the endogenous SERPINA1 site correlated to the amount of edited protein in circulation. These data support utilizing RNA editing to achieve normal A1AT protein levels to potentially alleviate the increased lung and liver disease risks seen in AATD patients.

BEAM-302 decreases hepatic aggregates of mutant AAT and increases circulating functional AAT in rodent models of Alpha-1 Antitrypsin Deficiency

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Alpha-1 antitrypsin deficiency (AATD) is an inherited disorder caused by mutations in the SERPINA1 gene that encodes an anti-protease, Alpha-1 Antitrypsin (AAT). The PiZ mutation, a G-to-A polymorphism in the SERPINA1 gene, is the most common mutation associated with severe AATD. Mutant AAT (Z-AAT) misfolds and forms aggregates that are proteotoxic to the liver. The inefficient secretion of Z-AAT leads to a deficiency of circulating AAT that can result in lung damage due to unopposed elastase activity. The PiZ mutation is an ideal target for correction to wildtype (PiM) by an adenine base editor (ABE) which converts an A to G in genomic DNA. BEAM-302 is a lipid nanoparticle (LNP) formulation of an mRNA encoding an ABE and a guide RNA (gRNA) targeting correction of the PiZ mutation. The pharmacological activity of BEAM-302 was characterized in the NSG-PiZ mouse model of AATD that carries multiple copies of the human PiZ transgene and demonstrates liver aggregates of Z-AAT. BEAM-302 was also evaluated in a novel rat model (PiZ rat) with a 1:1 replacement of rat AAT with human Z-AAT. In both models, a single, systemic administration of BEAM-302 induced dose-dependent rates of editing in liver. Base editing was associated with decreased Z-AAT aggregates in NSG-PiZ mouse livers and corresponding increases in circulating functional AAT in both models. Furthermore, repeat administration of BEAM-302 resulted in further increases in editing rates. Taken together these data support the hypothesis that base-editing by BEAM-302 has the potential to mitigate both the liver and lung pathology of AATD.

Developing therapeutics for AATD associated liver disease - progress in Takeda's clinical program

Ed G. Marins

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Alpha-1 antitrypsin (AAT) is a protein that is mainly produced in the liver, secreted into circulation and functions to counter the activity of neutrophil elastase in the lungs, protecting the tissue from elastase degradation (1,2). Over 100 mutations are described to affect the SERPINA-1 gene that encodes AAT, of which Pi*Z and Pi*S are the most common. Patients with Pi*ZZ genotype are at the highest risk of developing liver disease and most are distributed across North America, Europe, and Australia.

The Pi*Z allele encodes a misfolded AAT protein (Z-AAT) which is prone to polymerization and subsequent accumulation in the hepatocytes. The aggregated protein exceeds the protein clearance mechanism of hepatocytes creating an imbalance between protein production and degradation. This triggers a continuous cycle of inflammation and apoptosis with chronic regeneration leading to liver impairment, fibrosis, cirrhosis, and end-stage liver disease and in some cases the development of hepatocellular carcinoma. AAT aggregation in the liver also impedes its secretion into the circulation leading to serum levels below the threshold required to protect the lungs from neutrophil elastase, which also increases the risk of lung injury.

There seems to be a direct relationship between the accumulation of protein in the liver and fibrosis stage in adults with AATD associated liver disease where patients with higher accumulation levels showing more advance fibrosis than those with lower levels of protein3,4. This correlation seems to also hold true for survival whereby patients with higher accumulation having shorter survival as compared with those with lower accumulation (3,4).

The physiopathology of the disease provides a strong rationale for the development of fazirsiran (TAK-999), a drug under investigation for the treatment of AAT associated liver disease. Fazirsiran is a GalNac-conjugated small interfering RNA designed to silence the SERPINA-1 gene expression and decrease the production of Z-AAT. The drug demonstrates liver-selectivity and promotes the activation of the RNA-induced silencing complex targeting the AAT messenger RNA preventing its translation and subsequent protein accumulation.

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Drug Approval in Rare Disease: Liver Disease in Alpha-1 Antitrypsin Deficiency

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This presentation is intended to introduce regulatory concepts of developing drugs for treatment of rare disease, demonstrating clinical benefit, and use of surrogate endpoints to support approval of drug therapy. The presentation will also include a brief discussion of trial considerations for treatment of liver disease in alpha-1 antitrypsin deficiency.

The Critical Path for Alpha-1 antitrypsin deficiency (CPA-1) Consortium: a public-private collaborative approach to develop solutions for unmet needs specific to rare disease drug development

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There is a growing need for global collaboration to address unmet medical product development for rare diseases. Alpha-1 antitrypsin deficiency (AATD) is a clinically under-recognized genetic disorder with increased risk of chronic liver and lung diseases. AATD has been globally researched and advocated for, for over three decades with little global progress seen in the way of drug development and treatment options. Despite a very engaged global patient community with well-funded advocacy organizations and country specific registries, there remains significant unmet needs and gaps in knowledge that challenge the drug developers in this space. For this reason, the Critical Path Institute (C-Path) was engaged by the US Food and Drug Administration (FDA) to help bring together the various stakeholders from pharmaceutical industry, academic researchers, and patient groups to articulate the challenges, unmet needs, current literature and data available, and explore what opportunities may exist to advance a pathway forward for novel drug development for AATD.
In November 2021, C-Path and FDA began discussions to explore the feasibility of a sustainable public-private partnership dedicated to addressing drug development needs and challenges specific to AATD. Through support from FDA, the AATD pre-consortium effort began with a landscape analysis to specifically look at AATD drug development unmet needs. The pre-consortium effort brings together stakeholders from pharmaceutical companies, academic institutions, patient groups, non-government agencies, and regulatory agencies to evaluate key elements of the proposed ecosystem of AATD and put forth new ideas. Through the stakeholders' collective input and feedback, the group would then determine the high-level goals and objectives of the to-be-formed multi-stakeholder consortium whose objective would be to complete the design of the ecosystem. The collective input gathered during this pre-consortium effort would provide the basis for the formation of a sustainable consortium to address the specific needs of the AATD community. Collectively, the stakeholders will review the proposed ecosystem framework to ensure it could be effective in accelerating AATD medical product development and withstand regulatory review. The outcome of this pre-consortium effort is to launch a sustainable consortium, named Critical Path for Alpha-1 antitrypsin deficiency (CPA-1) Consortium, to address drug development unmet needs and actionable solutions through a public-private partnership approach with identified regulatory framework to generate drug development tools that receive endorsement from global regulatory bodies. This presentation provides a summary of the pre-consortium work, highlight the research identified by AATD stakeholders, and outline the C-Path model for data aggregation and analysis. The envisioned CPA-1 consortium aims to improve the lives of patients living with AATD and launches in late 2023.

Cell specific gene editing for treatment of Alfa 1 Antitrypsin Deficiency

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Alpha1 antitrypsin (AAT) deficiency (AATD), an autosomal codominant disease that is caused by mutation of the SERPINA1 gene, leads to liver and lung disease. Individuals with AAT deficiency usually develop symptoms of lung involvement at older ages, which include shortness of breath, chronic bronchitis, high prevalence of bronchial obstruction with Forced expiratory volume (FEV1) decline, and an ongoing decrease of computer tomography CT lung density that reflects progressive emphysematous lung destruction. The standard of care relies on weekly intravenous augmentation therapy with plasma-purified human AAT.

Our aim is to test a novel approach to obtain sustained therapeutic levels of circulating AAT using non-viral targeted cell specific base editing (BE) for hepatocytes. We will investigate whether this approach could permanently alter or attenuate the course of the disease. To test our hypothesis, we use cell lines as in vitro system and we will use pallid mice as animal model. Pallid mice carry a genetic deficiency of murine AAT, developing spontaneous emphysema at 8 months of age. Wild type (C57BL6) will serve as control.

Protospacer sequence (PAM) is designed for DNA base editing that specifically recognize the mutation present in pallid mice to enable editing the pathogenic base pair. PAM is encapsulated in cell specific liposomes as nano-carriers of about 100 nm in diameter to specifically target hepatocytes. We have standardized production, storage and engineering of liposomes. We have demonstrated, in vitro and in vivo, that our liposomes are cell specific.

This study will help us establish targeted cell specific non-viral BE method that can be used safely for gene editing in vivo. Non-viral cell specific gene editing can be further translated for clinical application as a promising therapeutic option for patients with AATD.

Multimodal decoding of human liver regeneration

Neil Henderson

University of Edinburgh, UK

To advance our understanding of human liver regeneration and fibrosis and to inform design of pro-regenerative and anti-fibrotic therapies, we have used multimodal single cell genomics approaches to generate a single-cell, pan-lineage atlas of human liver regeneration. In this seminar I will discuss how these approaches, in conjunction with functional interrogation of corollary cellular subpopulations in mouse models of liver regeneration and fibrosis, have allowed us to uncover novel cell states and unanticipated aspects of liver injury and repair.

Transcriptional induction of ER-phagy enhances lysosomal clearance of ATZ

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The endoplasmic reticulum (ER) is the largest mammalian intramembranous organelle. Engaged in multiple core functions, it executes structural, biosynthetic and signaling roles. Hence, it is not surprising that ER dysfunction is associated with the pathogenesis of several human diseases, ranging from rare genetic disorders to metabolic syndromes and cancer. Therefore, the identification of mechanisms controlling ER function could potentially shed light on the biological processes underpinning cellular health.
Very recently, the regulation of ER size was shown to be controlled by a selective receptor-mediated form of macro-autophagy, known as ER-phagy. During ER-phagy, ER fragments are sequestered into double membrane autophagosomes and subsequently degraded by lysosomes upon autophagosome-lysosome fusion. Importantly, ER-phagy exerts ER quality control functions by degrading excess/misfolded proteins that accumulate in the ER lumen including mutant ATZ and collagen molecules. We have discovered molecular mechanisms and signaling pathways that control ER-phagy activation in response to the accumulation of misfolded proteins in the ER lumen. Furthermore, by combining bioinformatic analysis with high content screening approaches we have identified novel pharmacological inducers of ER-phagy. Proof-of-concept experiments using osteogenesis imperfecta and alpha-1 antitrypsin deficiency primary cells demonstrate that pharmacological modulation of ER-phagy represents a potential novel therapeutic approach to counteract cellular dysfunction in diseases characterized by ER-dysfunction.

Z-α1-antitrypsin polymers impose molecular filtration in the endoplasmic reticulum after undergoing phase transition to a solid state

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Misfolding of secretory proteins in the endoplasmic reticulum (ER) features in many human diseases. In α1-antitrypsin deficiency, the pathogenic Z variant aberrantly assembles into polymers in the hepatocyte ER, leading to cirrhosis. We show that α1-antitrypsin polymers undergo a liquid:solid phase transition, forming a protein matrix that retards mobility of ER proteins by size-dependent molecular filtration. The Z-α1-antitrypsin phase transition is promoted during ER stress by an ATF6-mediated unfolded protein response. Furthermore, the ER chaperone calreticulin promotes Z-α1-antitrypsin solidification and increases protein matrix stiffness. Single-particle tracking reveals that solidification initiates in cells with normal ER morphology, previously assumed to represent a healthy pool. We show that Z-α1-antitrypsin–induced hypersensitivity to ER stress can be explained by immobilization of ER chaperones within the polymer matrix. This previously unidentified mechanism of ER dysfunction provides a template for understanding a diverse group of related proteinopathies and identifies ER chaperones as potential therapeutic targets.

Increased expression or activation of TRPML1 reduces hepatic storage of toxic Z alpha-1 antitrypsin

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Mutant Z alpha-1 antitrypsin (ATZ) accumulates in globules in the liver and is the prototype of proteotoxic hepatic disease. Therapeutic strategies aiming at clearance of polymeric ATZ are needed. Transient receptor potential mucolipin-1 (TRPML1) is a lysosomal Ca2+ channel that maintains lysosomal homeostasis. In this study, we show that by increasing lysosomal exocytosis, TRPML1 gene transfer or small-molecule-mediated activation of TRPML1 reduces hepatic ATZ globules and fibrosis in PiZ transgenic mice that express the human ATZ. ATZ globule clearance induced by TRPML1 occurred without increase in autophagy or nuclear translocation of TFEB. Our results show that targeting TRPML1 and lysosomal exocytosis is a novel approach for treatment of the liver disease due to ATZ and potentially other diseases due to proteotoxic liver storage.

Mitochondrial dysfunction in liver disease associated with AAT deficiency

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AAT deficiency (AATD) is a genetic cause of lung and liver disease and mainly affects patients carrying the Z allele of AAT (ATZ). Hepatocyte damage caused by endoplasmatic reticulum retention of ATZ also involves mitochondria and mitochondrial injury and authophagy (mitophagy) have been described in mouse and human livers expressing ATZ. However, their role in the pathogenesis of liver disease has not been investigated so far.

Peroxisome proliferator-activated receptor-coactivator type 1α (PGC-1α) is a master regulator of mitochondrial biogenesis. In ATZ transgenic PiZ mice we found chronic hepatic downregulation of PGC-1α and of its target genes involved in mitochondrial biogenesis and fatty acid catabolism. We hypothesize that repression of PGC-1α hampers the restoration of healthy mitochondrial pool and results in inefficient lipid catabolism and liver steatosis, a common feature of liver disease associated to AATD. We also found an imbalance of mitochondrial dynamics, characterized by excessive fission and reduced fusion. Alteration of mitochondrial dynamics may impair degradation of damaged mitochondria by autophagy and lead to the accumulation of malfunctioning mitochondria. Extracellular release of molecular danger signals from injured mitochondria triggers inflammation and fibrosis and may aggravate liver injury in response to ATZ accumulation. Consistently, we found an increase of circulating mtDNA during liver fibrosis development in PiZ mice.

In conclusion, these preliminary data suggest that mitochondrial dysfunction represents not only a consequence of hepatic ATZ accumulation but may be a driver of liver disease development and progression of in AATD.

Evaluation of polymer fate in ex vivo patient-derived organoids expressing Z alpha-1 antitrypsin

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Organoids are tiny, self-organized 3D tissue cultures crafted to replicate much of the complexity of an organ or to express selected features of it. Generally derived from stem cells, organoids represent an upgrade from the traditional primary cultures grown. Through their ability to differentiate and self-organizing mimicking tissue architecture, this model has a strong potential for the study of cellular dynamics in complex tissues and the development and characterisation of small molecules. In this work, we prove how to derive hepatic organoids expressing Z alpha-1 antitrypsin (AAT Z) directly from a transplanted liver assessing its biochemical properties in a novel patient-specific 3D model. A section of the liver was enzymatically dissociated at single cells, hepatocytes embedded in Matrigel to form 3D organoids and further expanded and differentiated in presence or absence of the polymer inhibitor GSK716. The biochemical characterization of these cells showed a solid consistency with previously observed values, confirming the kinetics of intracellular accumulation and secretion of AAT Z and the effect of GSK716 in inhibiting polymer formation. We believe that this new method for the direct formation of patient-specific organoids will represent an indispensable source of information for understanding and tuning the pathophysiological mechanisms of AAT deficiency.

Intercepting a structural intermediate of alpha1-antitrypsin on the path to polymer formation

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In its active state, alpha1-antitrypsin is in a kinetically stable, but thermodynamically unstable, configuration, rendering it susceptible to inappropriate conformational change. The alpha1-antitrypsin deposits that form in the liver of individuals with at least one Z allele are the consequence of an 'ordered aggregation' that yields linear, unbranched protein chains, termed polymers, that are both extremely stable and functionally inactive. Our aim is to define the molecular details of the polymerisation pathway, in which alpha1-antitrypsin passes through different conformational states as it transitions from a near-native monomer via multiple structural intermediates to a hyperstable polymeric form. We have used structural and biophysical methods, conformationally-selective monoclonal antibodies and small molecules to probe the structural and energetic aspects of the pathway. The antibody epitopes and ligand binding sites have been determined by integrating X-ray crystallography, SAXS, electron microscopy, conjugation of thiol reactive probes and molecular modelling. From the biophysical and spectroscopic measures of binding combined with physical characteristics of the intermediates and their relation to the terminal polymeric state, contextualised with the work of others, I will present the details of this pathway that are being revealed.

The gut-liver axis in AATD-associated liver disease

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Alpha-1-Antitrypsin (AAT) Deficiency (AATD) is an inherited genetic disorder caused by mutations in the SERPINA1 gene. The most severe AAT deficient allele is the Z variant, which leads to improper protein folding causing retention of the polymeric ATZ protein in the hepatocytes and inducing a variety of hepatic disorders ranging from fibrosis, cirrhosis, and increased risk of hepatocarcinoma.
Patients suffer from different onset and severity of the liver disease; of note, most of the patients with the homozygous ATZ mutation will never develop any liver disease. The reason for this phenotypic variability is unknown. Several studies have been carried out to investigate the involvement of genetic and environmental modifiers and their influence on the expression and severity of the liver disease among patients.
The aim of our study is to investigate the gut-liver axis in AATD, focusing in particular on the gut functionality and the possible connection between AATD-associated liver disease and the gut microbiota as modifier of the liver phenotype.
We found high human ATZ mRNA and protein levels in the intestine of PiZ mice, particularly in the ileum, the region of the small intestine where most of the microbes reside. Single cells RNA-sequencing analysis revealed that hATZ mRNA is expressed in all the intestinal cell types. We confirmed this data by co-immunostaining for hATZ and cell-specific markers. Of note, we observed a high number of hATZ polymers in Goblet, Enteroendocrine and Paneth cells, responsible for the secretion of the gut protective mucus layer, of hormones reflecting dietary intake, of factors necessary for stem cell function, and molecules with antimicrobial properties. Electron microscopy analysis confirmed accumulation of ATZ in Paneth cells leading to ER stress, reduction of lysozyme granules and subsequent cell death.
Finally, metagenomic analyses showed that WT and PiZ mice have a well distinguished intestinal microbiota composition. Importantly, we observed a more severe phenotype in male PiZ mice, which show overall microbial depletion, with many bacterial strains up- or down-represented as showed for many other liver diseases.
In conclusion our data suggest that hATZ expression and accumulation in intestinal cells may affect the gut and its microbiota, thus affecting the continuous bidirectional crosstalk between gut and liver. Altered gut-liver axis may also contribute to the different onset and severity of liver manifestation in AATD patients.

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Modeling MZ heterozygosity using patient-derived pluripotent stem cells

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Background: Alpha-1 antitrypsin deficiency is a common inherited cause of chronic liver disease driven by accumulation of misfolded protein aggregates and associated deleterious effects. It has long been recognized that PiZZ homozygous individuals are at increased risk for chronic liver disease. Whether some degree of increased risk is associated with the heterozygous state has only recently become clear. While older studies generated conflicting results, more recent evidence has identified a modest increased risk for clinically significant liver disease among heterozygous PiMZ individuals, particularly in the context of a second injury. The lack of a model system that faithfully reproduces human MZ hepatocyte biology hindered the determination of whether Z heterozygosity can induce injury in human hepatocytes as well as a direct comparison to the better-characterized effect of Z homozygosity. These studies extend this work to directly test the impact of Z heterozygosity on hepatocyte biology using genetically controlled syngeneic MZ and MM iHeps generated from three distinct ZZ patient-specific iPSC lines.
Hypothesis: Expression of a single Z allele is sufficient to promote liver injury in patient-derived hepatic cells.
Methods: We delivered CRISPR/Cas9 in combination with single stranded oligodeoxynucleotide donor templates to correct either one or both copies of the SERPINA1 Z mutation in iPSCs generated from patients homozygous for the Z mutation. Following directed differentiation, syngeneic ZZ, MZ, and MM iHeps were analyzed to identify the consequences of expression of either one or two copies of the Z mutation in cells that were otherwise genetically homogenous.
Results: Our experiments determined that a single mutant Z allele is sufficient to alter intracellular AAT trafficking and secretion in patient-derived iHeps. This dysregulation was sufficient to induce transcriptional ER stress and metabolic dysregulation characterized by perturbation of the metabolome and impaired mitochondrial function in ZAAT-expressing cells. We further observed heterogeneity in gene expression including the activation of specific UPR branches among MZ and ZZ but not MM iHeps.
Conclusion: We find that MZ iHeps exhibit a cellular phenotype intermediate to genetically matched ZZ and MM comparators.

Host preconditioning and transient mitogen expression via mRNA-LNP lead to robust primary human hepatocyte engraftment and iPSC-derived hepatocyte-like cell survival in mice

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Liver transplantation is the treatment for end stage liver disease, though donor organs are quite scarce. Alternatively, transplantation of healthy liver cells is a promising approach to restore liver function, either cell therapies with primary human hepatocytes (PHH) or induced pluripotent stem cell (iPSC) derived hepatocyte-like cells (HLC). PHH transplantation has been validated as safe in human, though major challenges that remain are low efficiency and lack of sustained benefit. HLC transplantation remains entirely preclinical, and factors that limit HLC engraftment in liver disease mouse models include poor survival, proliferation, and maturation of transplanted cells. We hypothesize that stimulating key regenerative pathways in transplanted hepatocytes using hepatocyte growth factor (HGF) and epidermal growth factor (EGF) and preconditioning the host liver with expression of the cell cycle inhibitor p21 to prevent host hepatocyte proliferation will improve survival, proliferation, and engraftment of PHHs and HLCs in an injured mouse liver. We established a safe way to transiently express HGF and EGF specifically in the liver using nonintegrative nucleoside-modified mRNA encapsulated in lipid nanoparticles (mRNA-LNP). We use AAV8-TBG-p21 to precondition the host mouse liver with long lasting p21 expression specifically in hepatocytes. NSG-PiZ mice serve as our injury model, recapitulating alpha-1 antitrypsin deficiency (AATD) associated liver disease. We find that both preconditioning the host mouse liver with AAV8-TBG-p21 and the HGF and EGF mRNA-LNP treatments significantly augment transplanted PHH survival and proliferation in vivo in NSG-PiZ mice, evidenced by histological quantification of transplanted cells and human albumin levels in mouse sera in comparison to control. Combined treatment leads to robust repopulation of the mouse liver with functional human cells (~30%) and amelioration of AATD liver disease. Furthermore, this combined p21 and HGF+EGF treatment improves transplanted iPSC-derived HLC survival in NSG-PiZ mice. Thus, blocking host hepatocyte proliferation and promoting survival and proliferation in transplanted hepatocytes enhances PHH and HLC engraftment, showing promise for cell therapy to treat liver diseases.

This work is supported by:
- NIH NIDDK R01DK124361-01A1
- NIH NIDDK 1F31DK135378-01
- Boston University CTSI TL1 Pre-Doctoral Fellowship in Regenerative Medicine TL1TR001410
- March of Dimes Research Grant #6-FY14-530
- Alpha 1 Foundation Research Grant #640084

Precision-Cut Liver Slices: A Cutting-Edge Tool for Advancing Drug Discovery in Liver Diseases?

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Precision-Cut Liver Slices (PCLS) offer several advantages over traditional in vitro models and animal studies. They retain the intricate three-dimensional architecture of the liver, thereby closely resembling the in vivo liver microenvironment. PCLS can be prepared from various species, including humans, enabling translational research and improving the predictive value of preclinical studies. By comprehensively analyzing the benefits and challenges associated with PCLS, we can unlock their full potential and drive advancements in the development of effective therapies for liver diseases.

Alteration of lipid homeostasis in ZZ liver organoids revealed by lipidomic and transcriptomic analysis

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The Z-AAT mutation (Glu342Lys) causes Z-AAT polymerization and intrahepatic accumulation, which can result in hepatic alterations leading to steatosis, fibrosis, or cirrhosis. We have used liver organoids derived from patients carrying Z-AAT mutation and HepG2 cells stably overexpressing Z-AAT to investigate the relationship between the accumulation of Z-AAT polymers and the increase in intracellular lipids in hepatocytes. In addition, we performed a lipidomic analysis to determine the accumulated lipid species in our model, as well as a transcriptomic study to reveal differentially expressed genes. Our results show accumulation of Z-AAT protein together with an increase in lipid content in hepatocytes and specifically identified the lipid species more remarkably increased. In addition, transcriptomic analysis revealed genes related to lipid metabolism and transport. In conclusion, AAT polymers related to accumulation of intracellular lipids, which also associates with altered expression of genes of lipid metabolism and transport, suggesting that Z-AAT organoids are good a model to study the steatosis shown in AATD liver disease.

A comprehensive approach to characterize novel rare variants of alpha-1-antitrypsin

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Alpha-1-antitrypsin deficiency (AATD) is an under-diagnosed disorder associated with mutations in the SERPINA1 gene encoding alpha-1-antitrypsin (AAT). Besides the most common pathogenic variants S (E264V) and Z (E342K), many rarer genetic variants of AAT have been found in patients and in the general population. As the pathogenic significance of rare missense variants is often unclear purely on the base of clinical data, we integrate computational, biochemical and cellular studies to better define their associated risk of pulmonary and liver disease. Bioinformatic pathogenicity predictors proved useful in the initial assessment of newly identified amino acid substitutions of AAT. However, expression of the variants in mammalian cellular models is still required to determine their tendency to form intracellular polymers and their secretory deficiency. Biochemical studies on the purified proteins give further insights in the structural effects of the mutations. By this integrated approach, we have characterized several pathogenic rare variants affecting different regions of the AAT molecule and identified variants with non-classical behaviour. A further level of complexity is represented by the occurrence of most rare variants in compound heterozygosity with Z-AAT and the formation of hetero-polymers. These in vitro studies are required to define the pathogenic impact of newly discovered AAT variants and will contribute to improve diagnosis and clinical management of AATD patients with rare genotypes.

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